

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/EP05/007340

International filing date: 07 July 2005 (07.07.2005)

Document type: Certified copy of priority document

Document details: Country/Office: EP
Number: 04016519.3
Filing date: 14 July 2004 (14.07.2004)

Date of receipt at the International Bureau: 09 August 2005 (09.08.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

07.07.2005

Bescheinigung

Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

04016519.3

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk



Anmeldung Nr:
Application no.: 04016519.3
Demande no:

Anmeldetag:
Date of filing: 14.07.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

UCB Farchim S.A.
Z.I. Planchy
Chemin de Croix Blanche, 10
C.P. 411
CH-1630 Bulle
SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Pharmaceutical composition of piperazine derivatives

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s)
revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K31/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PL PT RO SE SI SK TR LI

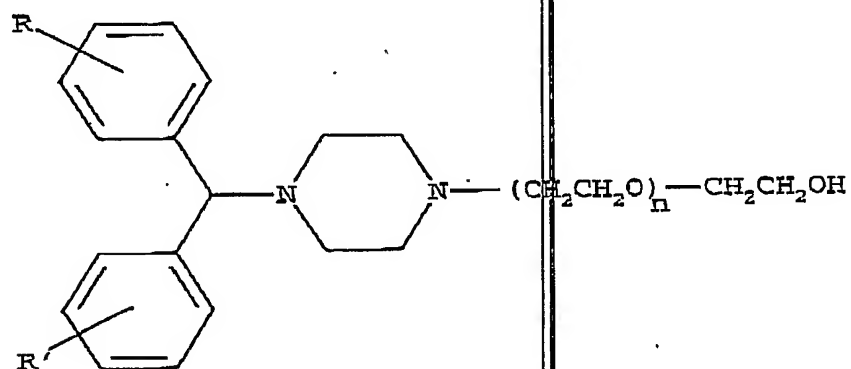
17.80.EP

Pharmaceutical composition of piperazine derivatives

The present invention relates to a liquid pharmaceutical composition containing an active substance belonging to the family of substituted benzhydryl piperazines.

A number of substances belonging to the family of substituted benzhydryl piperazines are known to be substances with useful pharmacological properties.

Patent GB 817231, for example, filed in the name of UCB, S.A., describes substituted benzhydryl piperazines having the general formula

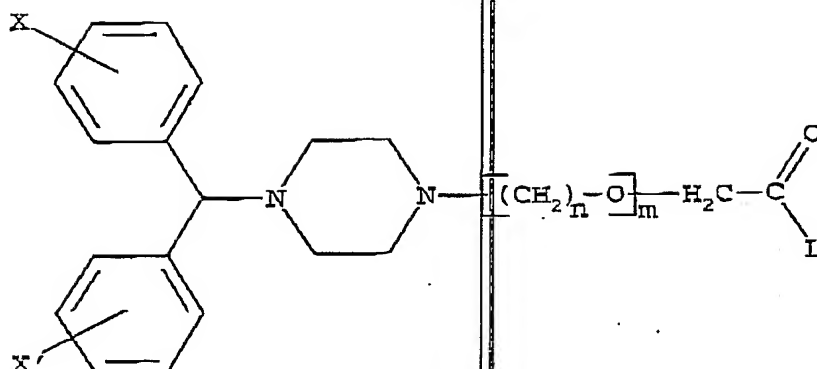


in which R and R¹ independently of one another represent a hydrogen or halogen atom, an alkyl or alkoxy group, it being possible for R and R¹ to be in the ortho, meta or para position, and n stands for the number 1 or 2, as well as their pharmaceutically acceptable salts.

In particular, these compounds include 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]ethanol, in particular, also known under the name of hydroxyzine, and its dichlorohydrate, which are well known for their antihistaminic and tranquillising properties.

Patent EP 58146, filed in the name of UCB, S.A., describes substituted benzhydryl piperazines having the general formula

2



in which L stands for an -OH or -NH₂ group. X and X', taken separately, stand for a
 5 hydrogen atom, a halogen atom, a linear or branched alkoxy radical at C₁ or C₄, or a
 trifluoromethyl radical, m equals 1 or 2, n equals 1 or 2, as well as their
 pharmaceutically acceptable salts.

Of these compounds, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-
 piperazinyl]ethoxy] acetic acid, also known under the name of cetirizine, and its
 10 dichlorohydrate are well known for their antihistaminic properties.

The active substances belonging to the family of substituted benzhydryl
 piperazines specifically include 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-
 piperazinyl]ethoxy]-acetic acid (cetirizine), 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-
 piperazinyl]ethoxy]ethanol (hydroxyzine), 2-[2-[4-[bis(4-fluorophenyl)methyl]-1-
 15 piperazinyl]ethoxy]acetic acid (efetirizine), 1-[(4-chlorophenyl)phenylmethyl]-4-[(3-
 methylphenyl)methyl]piperazine (meflazone) or 1-[(4-tert-butylphenyl)methyl]-4-[(4-
 chlorophenyl)phenylmethyl]piperazine (bucizine), their optically active isomers when
 applicable, as well as their pharmaceutically acceptable salts.

In the pharmaceutical field, solutions and drops are generally produced as
 20 germ-free compositions during their production processes. However, once the seal of
 the containers is broken, and the pharmaceutical compositions are completely used
 over a period of time, these pharmaceutical compositions are continuously exposed to
 the risk of being contaminated by the microorganisms existing in the environment or
 the human body, each time the containers are used and their covers are opened or
 25 closed.

It has now surprisingly been found that the active substances belonging to the
 family of substituted benzhydryl piperazines possess a preservative effect in aqueous
 solutions.

The purpose of the invention concerns a liquid pharmaceutical composition containing an active substance belonging to the family of substituted benzhydryl piperazines and a reduced amount of preservatives.

5 The present invention is based on the unexpected recognition that a pharmaceutical composition comprising an active substance belonging to the family of substituted benzhydryl piperazines and a reduced amount of preservatives is stable during a long period of time. Stability means the capacity to resist to microbial contamination.

10 The present invention encompasses a pharmaceutical composition comprising an active substance belonging to the family of substituted benzhydryl piperazines and an amount of parahydroxybenzoate esters used as preservatives less than 3 mg/ml of the composition, a normal concentration to preserve aqueous solutions.

15 The present invention encompasses a pharmaceutical composition comprising an active substance belonging to the family of substituted benzhydryl piperazines and at least one preservative, wherein the amount of preservative is in the case of parahydroxybenzoate esters more than 0 and less than 1.5 mg/ml of the composition, and in the case of other preservatives corresponds to the bactericidal effect of a parahydroxybenzoate esters concentration of more than 0 and less than 1.5 mg/ml.

20 Generally, the pharmaceutical composition of the invention is liquid and preferably aqueous.

By active substances belonging to the family of substituted benzhydryl piperazines, we understand also their optically active isomers and their pharmaceutically acceptable salts.

25 In the pharmaceutical composition of the invention, the active substance is generally selected from the group of cetirizine, levocetirizine, hydroxyzine, efletirizine, meclizine, buclizine, and their pharmaceutically acceptable salts. Preferably, the active substance is selected from the group of cetirizine, levocetirizine, and their pharmaceutically acceptable salts.

30 The term "cetirizine" refers to the racemate of [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid and its dihydrochloride salt which is well known as cetirizine dihydrochloride; its levorotatory and dextrorotatory enantiomers are known as levocetirizine and dextrocetirizine. Processes for preparing cetirizine, an individual optical isomer thereof or a pharmaceutically acceptable salt thereof have been described in European Patent 0 058 146, Great Britain Patent 2.225.320, Great Britain Patent 2.225.321, United States Patent 5,478,941, European Patent application 0 601 028, European Patent Application 0 801 064 and International Patent Application WO 97/37982.

The term "levocetirizine" as used herein means the levorotatory enantiomer of cetirizine. More precisely, it means that the active substance comprises at least 90% by weight, preferably at least 95% by weight, of one individual optical isomer of cetirizine and at most 10% by weight, preferably at most 5% by weight, of the other individual optical isomer of cetirizine. Each individual optical isomer may be obtained by conventional means, i.e., resolution from the corresponding racemic mixture or by asymmetric synthesis. Each individual optical isomer may be obtained from its racemic mixture by using conventional means such as disclosed in British patent application No. 2,225,321. Additionally, each individual optical isomer can be prepared from the racemic mixture by enzymatic biocatalytic resolution, such as disclosed in U.S. Patents No. 4,800,162 and 5,057,427.

The term "pharmaceutically acceptable salts" as used herein refers not only to addition salts with pharmaceutically acceptable non-toxic organic and inorganic acids, such as acetic, citric, maleic, succinic, ascorbic, hydrochloric, hydrobromic, sulfuric, and phosphoric acids and the like, but also its metal salts (for example sodium or potassium salts) or ammonium salts, the amine salts and the aminoacid salts. The best results have been obtained with dihydrochloride salts.

International patent application 94/06429 describes a method utilising levocetirizine for the treatment of allergic asthma.

Generally, the pharmaceutical composition of the invention contains an amount of preservatives selected in the range of 0.01 and 1.4 mg/ml of the composition. Preferably, it contains an amount of preservatives selected in the range of 0.2 and 1.125 mg/ml. More preferably it contains an amount of preservatives selected in the range of 0.3 and 1 mg/ml. The best results have been obtained with an amount of 0.375 to 0.75 mg/ml of the composition. The amount of the selected preservative is defined by comparison with the amount of parahydroxybenzoate ester leading to the same preservative effect. The optimum amount of preservative used in the invention depends on its nature. The preferred amount of preservative is such that it gives the same preservative effect as an amount of parahydroxybenzoate ester in the range of 0.2 and 1.125 mg/ml of the pharmaceutical composition.

By preservatives we understand a chemically substance that inhibits the development of microorganisms or, in an ideal instance, kills them; so antimicrobial agent able to limit or avoid the growth of microorganisms such as bacteria, yeast and moulds in a solution. Preservatives will comply with Eur P. and USP requirements: for a product incubated with a large number of bacteria and fungi, the preservative must kill and reduce a required amount of bacteria and fungi within a prescribed time period.

Examples of preservatives are p-hydroxybenzoate esters (methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, butyl parahydroxybenzoate, C1-C20 alkyl parahydroxybenzoate and their sodium salts), acrinol, methyl rosaniline chloride, benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, cetylpyrodium bromide, chlorohexidine, benzylalcohol, alcohol, chlorobutanol, isopropanol, ethanol, thimerosal, cresol, phenol, resorcin, sorbic acid, potassium and calcium sorbate, benzoic acid, potassium and calcium benzoate, sodium benzoate, calcium acetate, calcium disodium ethylenediaminetetraacetate, calcium propionate, calcium sorbate, diethyl pyrocarbonate, sulphur dioxide, sodium sulphite, sodium bisulfite, boric acid, sodium tetraborate, propionic acid, sodium and calcium propionate, nisin, sodium thiosulfate, or a mixture therefore. Generally, the preservative is selected from the group of p-hydroxybenzoate esters (methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, butyl parahydroxybenzoate, C1-C20 alkyl parahydroxybenzoate or a mixture thereof. Preferably the preservative is selected from the group of sodium benzoate, methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, a mixture of methyl parahydroxybenzoate and ethyl parahydroxybenzoate or propyl parahydroxybenzoate, and a mixture of methyl parahydroxybenzoate and propyl parahydroxybenzoate. Best results have been obtained with a mixture of methyl parahydroxybenzoate and propyl parahydroxybenzoate.

By patient, we understand children, adolescents and adults, preferably of 2 years old. The targeted patients are usually old from 2 years and more.

A preferred daily dosage provides from about 0,0005 mg to about 2 mg of levocetirizine or a pharmaceutically acceptable salt thereof, per kg of body weight per patient. A particularly preferred daily dosage is from about 0.001 to about 2 mg per kg of body weight per patient. The best results have been obtained with a daily dosage from about 0,005 to 1 mg per kg of body weight per patient. The dosage may be administered once per day of treatment, or divided into smaller dosages, for examples 1 to 4 times a day, and preferably 1 to 3 times a day, and administrated over about a 24 hours time period to reach a total given dosage. Bests results have been obtained with an administration of a composition of the invention twice a day for infants; and 5 mg once a day for children and adults. The exact dosages in which the compositions are administrated can vary according to the type of use, the mode of use, the requirements of the patient, as determined by a skilled practitioner. The exact dosage for a patient may be specifically adapted by a skilled person in view of the severity of the condition, the specific formulation used, and other drugs which may be involved.

The pharmaceutical forms according to the present invention may be prepared according to conventional methods used by pharmacists. The forms can be administered together with other components or biologically active agents, pharmaceutically acceptable surfactants, excipients, carriers, diluents and vehicles.

5 The pharmaceutical compositions of the invention include any conventional therapeutical inert carrier. The pharmaceutical compositions can contain inert as well as pharmacodynamically active additives. Liquid compositions can for example take the form of a sterile solution which is miscible with water. Furthermore, substances conventionally used as preserving, stabilizing, moisture-retaining, and emulsifying
10 agents as well as substances such as salts for varying the osmotic pressure, substances for varying pH such as buffers, and other additives can also be present. If desired an antioxidant can be included in the pharmaceutical compositions. Pharmaceutical acceptable excipients or carriers for compositions include saline, buffered saline, dextrose or water. Compositions may also comprise specific stabilizing
15 agents such as sugars, including mannose and mannitol. Carrier substances and diluents can be organic or inorganic substances, for example water, gelatine, lactose, starch, gum arabic, polyalkylene glycol and the like. A prerequisite is that all adjuvants and substances used in the manufacture of the pharmaceutical compositions are nontoxic.

20 Pharmaceutical compositions can be administered by spray inhalation. Any conventional pharmaceutical composition for spray inhalation administration may be used. Another preferred mode of administration is by aerosol.

The pharmaceutical compositions according to the present invention may also be administered orally. They may also be administered by nasal instillation, aerosols.
25 The pharmaceutical compositions which can be used for oral administration is liquid, for example, in the form of solutions, syrups, drops and the like.

The pharmaceutical forms, such as drops, nasal drops, are prepared by conventional pharmaceutical methods. The compounds of the present invention are mixed with a solid or liquid, non-toxic and pharmaceutically acceptable carrier and
30 possibly also mixed with a dispersing agent, a stabilizing agent and the like. If appropriate, it is also possible to add preservatives, sweeteners, coloring agents and the like.

Preferably, the pharmaceutical composition of the invention is administered in traditional form for oral administration, as oral liquid preparation such as syrup.

35 Best results have been obtained with an oral dosage form, in particular liquid formulations such as syrup for children.

An advantage of the invention is that reducing the concentration of the preservative leads to a reduction of the risk of an allergic reaction in sensitive patients.

7

Another advantage of the invention is the ability to make easier the manufacturing process avoiding the solubilization of important amounts of preservatives not freely soluble in water.

The invention is further defined by reference to the following examples.

5 Example 1. Preservative effect of cetirizine.

An oral solution and drops containing cetirizine were prepared. The compositions are given in table 1.

Table 1. - Cetirizine compositions

	Oral solution	Drops
10 Cetirizine hydrochloride (mg)	1	10
Sorbitol sol. At 70% (mg)	450	-
Glycerine (mg)	200	250
Propyleneglycol (mg)	50	350
Sodium saccharinate (mg)	1	10
15 Banana flavour (mg)	0.1754	-
Sodium acetate (mg)	4.2	10
Acetic acid	ad pH 5	ad pH 5
Purified water (ml)	ad 1	ad 1
20 The antimicrobial preservative effectiveness tests were realized according to the European Pharmacopoeia (Chap. 5.1.3.). Samples of the oral solution and the drops were inoculated with bacterial and yeast suspensions of <i>Pseudomonas aeruginosa</i> ATCC 9027, <i>Escherichia Coli</i> ATCC 8739, <i>Staphylococcus aureus</i> ATC C6538, <i>Candida albicans</i> ATCC10231 and <i>Aspergillus niger</i> ATCC16404. The number of viable		
25 microorganisms per ml of preparations under test were determined. The results are given in tables 2 and 3.		

Table 2. - Microbial content in inoculated sample of the oral solution

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
5					
Inoculum	5.5×10^5	4.6×10^5	4.0×10^5	3.7×10^5	2.3×10^5
0	4.9×10^5	4.7×10^5	3.1×10^5	2.6×10^5	1.7×10^6
7	< 100	< 100	< 100	< 100	4.8×10^5
14	< 1	< 1	< 1	2	8.2×10^3
21	< 1	< 1	< 1	< 1	5.5×10^3
10					
28	< 1	< 1	< 1	< 1	5.0×10^3

Table 3. - Microbial content in inoculated sample of the drops

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
15					
Inoculum	4.0×10^5	3.4×10^5	3.6×10^5	3.5×10^5	1.8×10^6
0	3.5×10^5	3.8×10^5	2.2×10^5	2.6×10^5	1.6×10^6
7	< 100	< 100	< 100	< 100	< 10^4
14	< 1	< 1	< 1	< 1	< 100
21	< 1	< 1	< 1	< 1	< 1
20					
28	< 1	< 1	< 1	< 1	< 1

In both cases, a rapid disappearance of *Pseudomonas aeruginosa*, *Escherichia Coli*, *Staphylococcus aureus* and *Candida albicans* is observed in the inoculated samples. For *Aspergillus niger*, the number of viable spores is significantly reduced in the oral solution while a rapid disappearance is observed in the drops.

Example 2. Preservative effect of levocetirizine.

An oral solution and drops containing levocetirizine were prepared. The compositions are given in table 4.

Table 4. – Levocetirizine compositions

	Oral solution	Drops
Levocetirizine hydrochloride (mg)	0.5	5
5 Maltitol-Lycasin 80-55 (mg)	400	-
Glycerine 85 %(mg)	235.2	294.1
Propyleneglycol (mg)	-	350
Sodium saccharinate (mg)	0.5	10
Tutti frutti flavour (mg)	0.15	-
10 Sodium acetate (mg)	3.4	5.7
Acetic acid (mg)	0.5	0.53
Purified water (ml)	ad 1	ad 1

- 15 The antimicrobial preservative effectiveness tests were realized according to the European Pharmacopoeia (Chap. 5.1.3.). Samples of the oral solution and the drops were inoculated with bacterial and yeast suspensions of *Pseudomonas aeruginosa* ATCC 9027, *Escherichia Coli* ATCC 8739, *Staphylococcus aureus* ATC C6538, *Candida albicans* ATCC10231 and *Aspergillus niger* ATCC16404. The number of viable microorganisms per ml of preparations under test were determined. The results are
- 20 given in tables 5 and 6.

Table 5. – Microbial content in inoculated sample of the oral solution

Time (days)	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
25 Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.2×10^5	1.8×10^5	3.5×10^5	3.9×10^5	1.6×10^6
7	150	< 100	< 100	2.8×10^4	1.0×10^3
14	< 1	< 1	< 1	1.4×10^4	4.8×10^5
21	< 1	< 1	< 1	2.6×10^2	2.2×10^5
30 28	< 1	< 1	< 1	6.2×10^3	5.3×10^5

Table 6. - Microbial content in inoculated sample of the drops

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
5 Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.2×10^5	1.5×10^5	3.1×10^5	1.8×10^5	1.7×10^6
7	< 100	< 100	< 100	< 100	9.0×10^4
14	< 1	< 1	< 1	< 1	< 1000
21	< 1	< 1	< 1	< 1	< 1
10 28	< 1	< 1	< 1	< 1	< 1

In both cases, a rapid disappearance of *Pseudomonas aeruginosa*, *Escherichia Coli*, *Staphylococcus aureus* is observed in the inoculated samples.

A disappearance of *Candida albicans* and *Aspergillus niger* is also observed in the drops.

Example 3. Efficacy of antimicrobial preservation of cetirizine aqueous solutions by p-hydroxybenzoate esters.

Oral solutions and drops containing cetirizine according to example 1 but also containing mixtures of p-hydroxybenzoate esters (methyl p-hydroxybenzoate/propyl p-hydroxybenzoate in a ratio of 9/1 expressed in weight). The total amounts of p-hydroxybenzoate esters were 0.15 mg/ml, 0.45 mg/ml, 0.75 mg/ml and 1.05 mg/ml. The efficacy of antimicrobial preservation of these solutions and drops was determined according to the European Pharmacopoeia (Chap. 5.1.3.). The results of the tests is given in tables 7 to 14.

Table 7. - Microbial content in inoculated sample of the oral solution containing 0.15 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
30 Inoculum	5.5×10^5	4.6×10^5	4.0×10^5	3.7×10^5	2.3×10^6
0	5.1×10^5	4.5×10^5	3.0×10^5	4.0×10^5	4.1×10^6
14	< 1	< 1	< 1	< 1	9.1×10^3
28	< 1	< 1	< 1	< 1	750

Table 8. - Microbial content in inoculated sample of the oral solution
containing 0.45 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
5					
Inoculum	5.5×10^5	4.6×10^5	4.0×10^5	3.7×10^5	2.3×10^6
0	5.2×10^5	4.9×10^5	3.3×10^5	2.9×10^5	1.2×10^6
14	< 1	< 1	< 1	< 1	<100
28	< 1	< 1	< 1	< 1	2

Table 9. - Microbial content in inoculated sample of the oral solution
containing 0.75 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
15					
Inoculum	5.5×10^5	4.6×10^5	4.0×10^5	3.7×10^5	2.3×10^6
0	3.9×10^5	4.4×10^5	4.0×10^5	1.9×10^5	1.9×10^6
14	< 1	< 1	< 1	< 1	<100
28	< 1	< 1	< 1	< 1	< 1

Table 10. - Microbial content in inoculated sample of the oral solution
containing 1.05 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
20					
Inoculum	5.5×10^5	4.6×10^5	4.0×10^5	3.7×10^5	2.3×10^6
25					
0	3.3×10^5	4.1×10^5	3.1×10^5	1.4×10^5	1.2×10^6
14	< 1	< 1	< 1	< 1	<100
28	< 1	< 1	< 1	< 1	< 1

Table 11. - Microbial content in inoculated sample of the drops
containing 0.15 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
30					
Inoculum	4.0×10^5	3.4×10^5	3.6×10^5	3.5×10^5	1.8×10^6
35					
0	4.3×10^5	4.0×10^5	2.0×10^5	2.5×10^5	1.5×10^6
14	< 1	< 1	< 1	< 1	<100
28	< 1	< 1	< 1	< 1	< 1

12

Table 12. - Microbial content in inoculated sample of the drops containing 0.45 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
5					
Inoculum	4.0×10^5	3.4×10^5	3.6×10^5	3.5×10^5	1.8×10^6
0	3.6×10^5	3.6×10^5	1.7×10^5	2.1×10^5	1.4×10^6
14	< 1	< 1	< 1	< 1	< 100
28	< 1	< 1	< 1	< 1	< 1

Table 13. - Microbial content in inoculated sample of the drops containing 0.75 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
10					
Inoculum	4.0×10^5	3.4×10^5	3.6×10^5	3.5×10^5	1.8×10^6
0	4.1×10^5	3.6×10^5	2.6×10^5	2.5×10^5	1.6×10^6
14	< 1	< 1	< 1	< 1	< 100
28	< 1	< 1	< 1	< 1	< 1

Table 14. - Microbial content in inoculated sample of the drops containing 1.05 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
20					
Inoculum	4.0×10^5	3.4×10^5	3.6×10^5	3.5×10^5	1.8×10^6
0	3.9×10^5	3.7×10^5	2.8×10^5	2.2×10^5	1.3×10^6
14	< 1	< 1	< 1	< 1	< 100
28	< 1	< 1	< 1	< 1	< 1

In all cases, the disappearance of Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Candida albicans is observed in the inoculated samples.

30 For Aspergillus niger, the number of viable spores is significantly reduced in the oral solution while a rapid disappearance is observed in the drops.

In all cases the recommended efficacy criteria were achieved.

Example 4. Efficacy of antimicrobial preservation of levocetirizine aqueous solutions by p-hydroxybenzoate esters.

35 Oral solutions and drops containing levocetirizine according to example 2 but also containing mixtures of p-hydroxybenzoate esters (methyl p-hydroxybenzoate/propyl p-hydroxybenzoate in a ratio of 9/1 expressed in weight). The total amounts of p-

hydroxybenzoate esters were 0.375 mg/ml, 0.75 mg/ml and 1.125 mg/ml. The efficacy of antimicrobial preservation of these solutions and drops was determined according to the European Pharmacopoeia (Chap. 5.1.3.). The results of the tests is given in tables 15 to 20.

5

Table 15. – Microbial content in inoculated sample of the oral solution containing 0.375 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
10 Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.7×10^5	1.3×10^5	2.8×10^5	3.8×10^5	1.6×10^6
14	< 1	< 1	< 1	1.7×10^4	1.6×10^5
28	< 1	< 1	< 1	< 1	< 100

15

Table 16. – Microbial content in inoculated sample of the oral solution containing 0.75 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
20 Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.5×10^5	1.6×10^5	2.4×10^5	3.4×10^5	1.6×10^6
14	< 1	< 1	< 1	5.5×10^2	1.4×10^4
28	< 1	< 1	< 1	< 1	< 1

25

Table 17. – Microbial content in inoculated sample of the oral solution containing 1.125 mg/ml of p-hydroxybenzoate esters

Time (days)	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Candida albicans	Aspergillus niger
30 Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.9×10^5	1.2×10^5	3.0×10^5	3.5×10^5	1.4×10^6
14	< 1	< 1	< 1	< 10	< 1000
28	< 1	< 1	< 1	< 1	< 1

14

Table 18. - Microbial content in inoculated sample of the drops
containing 0.375 mg/ml of p-hydroxybenzoate esters

Time (days)	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Escherichia</i> <i>coli</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Candida</i> <i>albicans</i>	<i>Aspergillus</i> <i>niger</i>
5					
Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.1×10^5	1.2×10^5	2.6×10^5	1.7×10^5	1.8×10^6
14	< 1	< 1	< 1	< 1	< 1000
28	< 1	< 1	< 1	< 1	< 1

10

Table 19. - Microbial content in inoculated sample of the drops
containing 0.75 mg/ml of p-hydroxybenzoate esters

Time (days)	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Escherichia</i> <i>coli</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Candida</i> <i>albicans</i>	<i>Aspergillus</i> <i>niger</i>
15					
Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	3.1×10^5	1.0×10^5	3.0×10^5	1.8×10^5	1.4×10^6
14	< 1	< 1	< 1	< 1	< 1000
28	< 1	< 1	< 1	< 1	< 1

20

Table 20. - Microbial content in inoculated sample of the drops
containing 1.125 mg/ml of p-hydroxybenzoate esters

Time (days)	<i>Pseudomonas</i> <i>aeruginosa</i>	<i>Escherichia</i> <i>coli</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Candida</i> <i>albicans</i>	<i>Aspergillus</i> <i>niger</i>
25					
Inoculum	3.6×10^5	1.7×10^5	2.7×10^5	3.4×10^5	1.7×10^6
0	2.9×10^5	6.9×10^4	2.7×10^5	5.0×10^4	1.5×10^6
14	< 1	< 1	< 1	< 1	< 1000
28	< 1	< 1	< 1	< 1	< 1

In all cases, the disappearance of *Pseudomonas aeruginosa*, *Escherichia Coli*,

30 *Staphylococcus aureus* and *Candida albicans* is observed in the inoculated samples.

For *Aspergillus niger*, the number of viable spores is significantly reduced in the oral solution while a rapid disappearance is observed in the drops.

In all cases the recommended efficacy criteria were achieved.

35

CLAIMS

1. A liquid pharmaceutical composition comprising an active substance belonging to the family of substituted benzhydryl piperazines and at least one preservative, wherein the amount of preservative is in the case of parahydroxybenzoate esters more than 0 and less than 1.5 mg/ml of the composition, and in the case of other preservatives corresponds to the bactericidal effect of a parahydroxybenzoate esters concentration of more than 0 and less than 1.5 mg/ml.
2. A liquid pharmaceutical composition according to claim 1, characterized in that it is an aqueous composition.
3. A liquid pharmaceutical composition according to claim 1 or 2, characterized in that the preservative is selected from the group of sodium benzoate, methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, a mixture of methyl parahydroxybenzoate and ethyl parahydroxybenzoate or propyl parahydroxybenzoate, and a mixture of methyl parahydroxybenzoate and propyl parahydroxybenzoate.
4. A liquid pharmaceutical composition according to claim 3, characterized in that the preservatives is a mixture of methyl parahydroxybenzoate and propyl parahydroxybenzoate.
5. A liquid pharmaceutical composition according to any of the preceding claims, characterized in that the pharmaceutical composition contains an amount of preservatives selected in the range of 0.01 and 1.4 mg/ml of the composition.
6. A liquid pharmaceutical composition according to claim 5, characterized in that the pharmaceutical composition contains an amount of preservatives selected in the range of 0.2 and 1.125 mg/ml.
7. A liquid pharmaceutical composition according to claim 6, characterized in that the pharmaceutical composition contains an amount of preservatives selected in the range of 0.3 and 1 mg/ml.
8. A liquid pharmaceutical composition according to claim 7, characterized in that the pharmaceutical composition contains an amount of preservatives selected in the range of 0.375 to 0.75 mg/ml of the composition.
9. A liquid pharmaceutical composition according to any of the preceding claims, characterized in that the active substance is cetirizine.
10. A liquid pharmaceutical composition according to any of the claims 1 to 8, characterized in that the active substance is levocetirizine.

1

ABSTRACT

The present invention relates to a liquid composition containing an active substance belonging to the family of substituted benzhydryl piperazines with reduced amounts of preservatives.

5